The spectrum of gluten related diseases

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Part III

Chapter 4

Refractory celiac disease and EATL patients show severe malnutrition and malabsorption at diagnosis

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Abstract

Background & Aims: Refractory celiac disease type II (RCDII) and EATL (Enteropathy Associated T-cell Lymphoma) are (pre)malignant complications of celiac disease (CD). Data on malnutrition and intestinal absorption is lacking in these patients. Therefore, the aim of the study is to comprehensively assess nutritional status and intestinal absorption capacity of patients with RCDII and EATL, compared with data of newly diagnosed CD patients.

Methods: Observational study in tertiary care setting in RCDII (n=24, 63.8±8.2y), EATL (n=25, 62.3±5.7y) and CD patients (n=43, 45.6±14.8y). At diagnosis, anthropometry (BMI, unintentional weight loss, fat-free mass index (FFMI), handgrip strength (HGS), nutritional intake, fecal losses and Resting Energy Expenditure (REE)) were assessed.

Results: Low BMI (<18.5) was more often observed in RCDII patients than in CD or EATL patients (in 33%, 12% and 12%, respectively, p=0.029). EATL patients more frequently had unintentional weight loss (>10%) than CD or RCDII patients (in 58%, 19% and 39% of patients, respectively; p=0.005/0.082). Energy malabsorption (<85%) was detected in 44% and 33% of RCDII and EATL patients, vs 21.6% in CD (NS). Fecal energy losses were higher in RCDII than in CD patients (589±451 vs 277±137 kcal/d, p=0.017). REE was underestimated by predicted-REE with>10% in 60% of RCDII, 89% of EATL, and 38% of CD patients (p=0.006). Low FFMI and HGS were detected in one third and two thirds of all patients, respectively.

Conclusions: The nutritional status of patients with RCDII and EATL is inferior compared with untreated naïve CD patients at presentation. Both malabsorption as well as hypermetabolism contribute to malnutrition.

Introduction

Celiac disease (CD) is defined as an immune-mediated chronic enteropathy, caused by an irreversible intolerance for gluten in individuals who are genetically susceptible. Its prevalence has been estimated to be 0.5%-1% (1). Histopathological characteristics comprise a variable degree of villous atrophy, crypt hyperplasia and intra-epithelial lymphocytosis, primarily in duodenum and jejunum (2, 3). The only accepted treatment is a strict and lifelong adherence to a gluten free diet (GFD), which interrupts the immune response triggered by gluten.

Most patients improve clinically within several weeks to months after instigation of a GFD (4). In a substantial number of patients mucosal recovery is delayed and may last until 2 years after initiation of a strict diet (5-8). A small minority of patients (approximately 0.5-1% of adulthood diagnosed CD (5)) fails to improve clinically upon a strict GFD for over 12 months (primary resistance) or shows a relapse (secondary resistance). The most common cause for refractoriness to a GFD is unintentional contamination with gluten (8) or an (associated) disorder of the small bowel resembling CD. When dietary adherence is meticulously evaluated by a skilled dietician and other reasons for villous atrophy have been ruled out, patients are diagnosed with refractory CD (RCD). RCD is subdivided into 2 types based on the non-presence (type I) or presence (type II) of abnormal intraepithelial lymphocytes (IELs) referred to as aberrant lymphocytes (9), the cut-off for RCDII being >20% (10). These 2 groups differ fundamentally since RCDII, in contrast to RCDI, may be considered as a low-grade lymphoma that may develop into a (destructive) enteropathy-associated-T-cell lymphoma (EATL) with an excessive mortality (11). Untreated, 60-80% of RCDII patients develop an EATL within 5 years. However, EATL may also develop in association with uncomplicated (secondary EATL) or unknown CD (primary EATL).

Symptoms in uncomplicated active CD patients may include diarrhea, abdominal pain, fatigue, malaise, deficiencies and weight loss, although clinical signs may also be succinct or even absent. Based on clinical presentation, RCDII and EATL patients show overlapping characteristics with ‘active’ or untreated CD; ongoing weight loss, diarrhea and fatty stools are usual and refractory to dietetic treatment, thus, adherence to a strict GFD (12, 13). Unsuitably, literature regarding other nutritional parameters and energy expenditure in both RCDII and EATL is lacking.

Since the pathophysiology and etiology of malnutrition are complex, it appears inappropriate to assess nutritional status and its possible determinants (e.g. malabsorption and increased metabolism) on the basis of a single parameter,

ignoring/neglecting temporal weight loss, Body Mass Index (BMI), body composition, functional indices, intestinal absorption and basal metabolism. Therefore, the aim of this study was to perform a comprehensive assessment of the nutritional status and energy balance of patients with RCDII and EATL and to compare these results with newly diagnosed, naive CD patients.

Materials and methods

This observational cross sectional study was performed in recently diagnosed, naive CD, RCDII and EATL patients. Nutritional status was determined according to three independent variables: 1) current BMI and percentage of weight loss (unintentionally) during the 6 months prior to diagnosis; 2) body composition and 3) a parameter of functionality being handgrip strength (HGS). Energy balance was determined evaluating nutritional intake, fecal losses and resting energy expenditure (REE). Energy balance (kcal/d) was calculated as the difference between energy intake and 'total energy use', the latter being calculated as Total Energy Expenditure (TEE) plus fecal energy loss. All measurements were performed in one routinely medical appointment by an experienced dietician.

Patients

During the period 2005-2013, all consecutively diagnosed adult RCDII or EATL patients from the outpatient clinic of the VU University Medical Centre, Amsterdam, the Netherlands, were enrolled. Besides, newly diagnosed (naïve) CD patients were concurrently and consecutively recruited. Patients either consumed a normal or standard Dutch western and gluten containing diet (newly diagnosed CD and primary EATL patients) or a GFD for at least 12 months prior to diagnosis (RCDII patients and EATL patients secondary to a former diagnosis of CD or RCDII).

Diagnosis of CD, RCDII and EATL

Anti-endomysial (EMA) and anti-transglutaminase antibodies (tTG), i.e. the CD associated antibodies, were determined. In addition, HLA-genotyping was determined, to analyze the incidence of DQ_2 and DQ_8 haplotypes as a requirement for a conclusive diagnosis. Duodenal biopsy specimens were gathered to define the grade of histological impairment as classified by Marsh (14) (modified by Rostami (15, 16)) the gold standard method of diagnosis of CD.

CD diagnosis relied on the demonstration of partial or complete villous atrophy (Marsh IIIA-C), and the detection of CD-related antibodies and the presence of CD-related genotypes. In the CD patient group, patients with low grade histopathological abnormalities (Marsh I or II, i.e. lymphocytic enteritis with crypt hyperplasia) could only and exceptionally be included in case of family screening, together with presence of gluten-dependent disorders, in combination with elevated CD associated antibodies and HLA-DQ_2 or HLA-DQ_8 haplotype.

The RCDII diagnosis was based on recurring or persisting clinical symptoms and villous atrophy of the small intestine (Marsh IIIA-C) which remained or reoccurred in spite of a strict GFD for over a year and at the exclusion of other villous atrophy causes. Furthermore, the clinically endorsed cut-off value of over 20% aberrant IELs (perceived by flow cytometric analysis) was used to diagnose RCDII (10). The EATL diagnosis was based on the criteria according to the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues. Diagnosis was confirmed histologically by our expert pathologist. Primary EATL was defined as diagnosis of EATL in patients without a preceding CD history or when diagnosis of both EATL and CD were made at the same time or with a maximal six-months-interval. Inconclusive or negative serology was not an exclusion criteria in all patients groups, when patients met the villous atrophy and CD-related genotype criteria.

The study protocol was approved by the Medical Ethics Committee of the VU University Medical Centre Amsterdam, The Netherlands. All patients provided informed consent.

Nutritional status

BMI and weight loss

Patient characteristics, demographic data and anthropometric data were collected directly after diagnosis and before dietary treatment to either initiate a GFD (CD patients) or nutritional support (RCD or EATL patients). Nutritional status parameters included body weight (in kg, measured on a digital electronic scale (with an accuracy of 0.1 kg), and self-reported body height (m) and weight loss (involuntary in kg) in the past one month and in the past six months. Patients were classified into three BMI cohorts (based on the World Health Organization's definition (2000)): BMI up to 18.5 kg/m^2 (underweight), 18.5-25.0 kg/m^2 (normal weight) and over 25 kg/m^2 (overweight, or 'obese' when BMI exceeded 30 kg/m^2). Patients were classified as 'malnourished' in case of unintentional loss of bodyweight of more than 10% in the past six months or more than 5% loss of body weight in the month previous to diagnosis. Patients were classified as having 'risk of

malnutrition' in case of 5%-10% loss of bodyweight (unintentionally) in the six months prior to diagnosis. Overall patients were classified as having a 'normal nutritional status' when BMI exceeded 18.5 and no weight loss (<10% in past six months or <5% in one month) occurred.

Body composition

Body composition was measured using (the 50 KHz data of) a multiple frequency bioelectrical impedance analyzer (Hydra ECF/ICF Bio-Impedance Spectrum Analyzer, model 4200, Xitron Technologies, San Diego, CA, USA). During the test, patients were placed in a supine position without socks and shoes. Four adhesive electrodes (3M Red Tod T, 3M Health care, Borken, Germany) were attached to the skin in a standard tetrapolar position; at the dorsal sides of the dominant hand and foot and at the distal metacarpals and metatarsals. Whole-body reactance and resistance were measured by an electronic current (50 kHz). The fat free mass (in kg and %, FFM) was calculated by the equation of Kyle (17). The fat free mass index (in kg/m^2, FFMI) was calculated as FFM (kg)/height^2 (in m). Cut-off values for FFMI were <16.7 FFM kg/m^2 for men, and <14.6 FFM kg/m^2 for women, respectively (17).

Handgrip strength

Handgrip strength of the non-dominant arm was measured using a Physio-Med Baseline Hydraulic handgrip dynamometer. During the test, patients were asked to sit in a relaxed position, facing the back of the dynamometer display. The arm which was tested was not supported during the test and the elbow was in 90 degrees flexion. The highest of three recorded measurements was taken into account in subsequent analysis. Age, gender, and sex specific reference values for hand grip strength were used, according to Bohannon (18). Values below the 10th percentile were considered as decreased (or too low).

Energy balance

Nutritional intake

During four consecutive days, patients were asked to report their nutritional (solid and liquids) intake in a nutritional diary. A skilled and experienced dietician instructed all patients beforehand regarding accurate weighing of all foods and beverages. The use of digital electronic scale was advised and specific dietary information was recorded,

including cooking methods and brand names, if any, for all foods and beverages consumed during the study period. Besides, the dietitian interviewed all patients afterwards to complete the records and to check whether all study procedures had meticulously been followed. A computerized food calculation program (based on the National Dutch Food Composition Table 'NEVO' 2006 (19), was applied to calculate nutrient intake (fat, protein and carbohydrates). The total dietary energy intake (TEI) was calculated using gross energetic values for the macronutrients, as described extensively elsewhere (3).

Fecal loss

All stools were collected during a period of exactly 72 hours (day 2-4 of study), as per protocol, in 5 liter fecal collection buckets. The patients were instructed to keep the feces stored in a dry and cool place during the collection period. Feces was weighed ('wet') upon arrival in the lab(in g/d, FWW), homogenized and instantly stored at <4°C till analysis. Feces was analyzed for energy, fat and nitrogen content, in order to calculate intestinal absorption capacity for these macronutrients. The fat content of the feces (F_{Fat}) was measured by the Van de Kamer method (21). The nitrogen content of the feces ($F_{Nitrogen}$), was analysed by the micro-Kjeldahl method, developed for wet stool samples, using formerly described digestive and catalytic conditions (22). Fecal protein content ($F_{Protein}$) was calculated using a fixed conversion factor, supposing that all of the fecal nitrogen content was a resultant of protein. In formula: $F_{Protein}$ (g/d) = $F_{Nitrogen}$ (g/d) x 6.25. The caloric value of $F_{Protein}$ was calculated as $F_{Protein}$ x 4.4 kcal/d. Next, a fecal sample was reserved, freeze dried and prepared to process by bomb calorimetry (as described elsewhere) (3, 23, 24). The fecal calorimetric determination represented the total daily energy loss by feces (F_{Energy}) in kcal/d. They were performed at the laboratory of University Medical Center Groningen, The Netherlands, with a Gallenkamp Ballistic bomb calorimeter (type CBB-33). Finally, the fecal 'rest' energy, i.e. the fecal energy content which does not reflect the caloric value of fecal-fat or fecal-protein, was defined as the fecal carbohydrate ($F_{Carbohydrate}$) content in this study. . In formula: $F_{Carbohydrate}$ (g/d) = (F_{Energy} - F_{Fat} x 9.4 - $F_{Protein}$ x 4.4) / 4.10. Subsequently, the intestinal absorption capacity of ingested energy from macronutrients (in %), was calculated as: (TEI - F_{Energy}/ TEI) x 100%. Malabsorption of energy or macronutrients (fat, protein and carbohydrate) was a priori determined as a cut-off value of < 85% and severe malabsorption as < 75% intestinal absorption (25, 26).

Energy Expenditure

Resting Energy Expenditure (REE) was assessed by indirect calorimetry (using Datex Deltatrac, Helsinki, Finland). The equipment was calibrated before each measurement, with calibration gas (95% oxygen and 5% carbon dioxide, Quick cal, GE Healthcare, Helsinki, Finland). Patients were instructed to lie in supine position and at complete rest when measurements were performed, throughout the total time of 30 minutes as a minimum. Steady state periods of measurements were selected, i.e. periods where carbon dioxide production and oxygen consumption were measured with a coefficient of variation beneath 10%. The Weir Equation was used by de Datex Deltatrec to calculate REE. Measured REE was compared to predicted REE according to the commonly used sex and age specific equation of Harris and Benedict (27). Measurements were classified as 'well estimated' by HB-prediction equation when measured REE fitted between 90 to 110% of predicted REE, as 'overestimated' when REE was less than 90% of predicted REE and 'underestimated' by HB-prediction equation when measured REE was more than 110% of predicted REE. The latter was, in this study, defined as 'hypermetabolism'. Moreover, REE was also presented corrected for bodyweight (in kcal/kg) and FFM (in kcal/kg FFM) despite different nutritional status between patient groups. Patients' Total Energy Expenditure (TEE) was calculated as measured REE + 30% for physical activity.

Statistical considerations

All data of the included patients are presented as means ± SD. Groups were compared with Students' *t*-test or analysis of variance (ANOVA) for continuous variables plus a Bonferroni correction once statistical significance was attained. Mann-Whitney U test / Wilcoxon or Kruskal-Wallis test, in case of more than two groups, were applied for continuous variables without a normal distribution. Pearson's correlation coefficient (*r*) and Chi Square test (for dichotomous variables and percentages) were used to study associations between variables, where appropriate. The level of statistical significance was set at $p<0.05$. The statistical analyses were performed by use of the software package SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, USA for Windows version 20).

Results

Patients

Ninety-two patients were included. Baseline characteristics of the total group and the three subgroups (CD n=43, RCDII n=24, EATL n=25) are presented in table 1. Of the 25 included EATL patients, 15 (60%) suffered from primary EATL, 4 patients (16%) presented with a secondary EATL following uncomplicated CD and 6 (24%) suffered from secondary EATL following RCDII. RCDII and EATL patients were statistical significantly older than CD patients at diagnosis.

Table 1: Patients characteristics (mean ± SD or N) of patients with RCDII and EATL versus CD at presentation

		CD (43)	RCDII (24)	EATL (25)	P-value (ANOVA)
Sex (N)		28F / 15M	11F / 13M	12F /13M	0.219
Age (year)		45.6±14.8	63.8±8.2	62.3±5.7	*<0.001* [I]
(range)		(21-75)	(45-78)	(51 -71)	
Height (m)		1.71±0.11	1.70±0.1	1.72±0.1	0.847I
Weight (kg)		69.2±16.3	60.9±10.6	64.0±9.9	*0.044* [II]
Marsh classification (N)	I/II [III]	6	1	2	
	IIIA	22	1	9	
	IIIB	8	9	8	
	IIIC	7	4	6	
Antibodies EMA/tTG(N)	negative	11	18	11	
	inconclusive	4	3	0	
	positive	27	1	14	
	n.d.	1	2	0	
% Aberrant cells		n.d.	60.1±23.6	26.1±31.6	<0.001[IV]
CD genotypes (N)	DQ_2 (heterozygote)	27	13	9	0.03 [v]
	DQ_2 (homozygote)	3	9	13	
	DQ_8 (heterozygote)	3	0	0	
	DQ_2 and DQ_8	2	1	2	
	DQ_2 nor DQ_8	2	1	0	
	n.d.	6	0	1	

[I] CD younger than RCDII and EATL, [II] RCDII lower weight than CD, [III] low grade histopathological abnormality plus HLA-DQ_2 and/or DQ_8 plus elevated CD associated antibodies (EMA and/or tTG), [IV] n.d. not determined, Student's *t*-test, , [v] Pearson Chi-square test

Nutritional status

Data on nutritional status are presented in table 2. Underweight (BMI <18.5 kg/m^2) was present in 16 patients, more often in RCDII patients than in naive CD or EATL patients (33.3 vs 12 and 12%, p=0.029). Moreover, mean BMI was lower in RCDII than in naïve CD patients (20.9 ±2.6 and 23.4±4.3 kg/m^2, p=0.012). Thirty-one patients of the total group were classified as malnourished having suffered >10% weight loss (unintentionally) in the past 6 months. EATL patients expressed, on average, the highest percentage unintentional weight loss (p=0.005). Moreover a higher proportion of patients in this group suffered from malnutrition (>10% unintentional weight loss) compared to naive CD or RCDII patients (58.3, 39.1 and 19.0%, respectively; p=0.001).

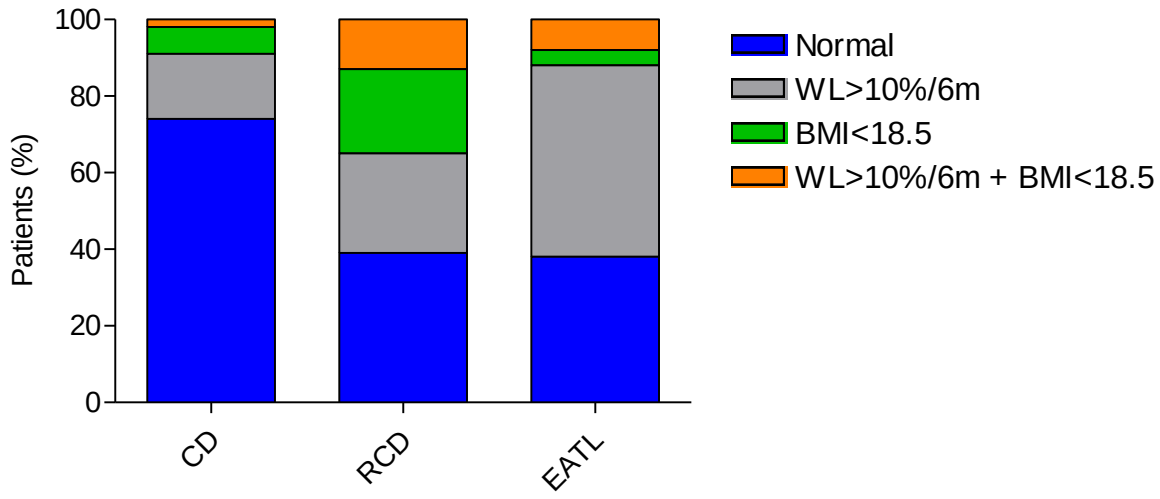
Table 2: Nutritional status (mean ± SD) of patients with RCDII and EATL versus uncomplicated CD at presentation

	CD novo (43)	RCDII (24)	EATL (25)	p-value
BMI (kg/m^2)	23.4 ± 4.3	20.9 ± 2.5	21.6 ± 2.6	*0.012* [I, V]
<18.5	11.6 %	33.3 %	12.0 %	*0.029* [VI]
18.5–25	58.1 %	62.5 %	72.0 %	
>25	30.2 %	4.2 %	16.0 %	
% Weight loss (N)	3.2 ± 6.1 (42)	6.5 ± 8.7 (23)	17.5 ± 30.4 (24)	*0.005* [II, V]
% malnourished [IV]	19.0	39.1	58.3	*0.001*[VI]
FFM [IV] (kg) (N)	47.4 ± 11.6 (34)	47.9 ± 10.5 (17)	48.8 ± 10.0 (15)	0.915 [V]
FFM (%)	71.0 ± 7.9	78.2 ± 14.1	75.6 ± 9.3	0.052 [III, V]
FM [IV] (kg)	19.5 ± 7.6	14.9 ± 7.1	15.7 ± 6.2	0.058 [III, V]
FM (%)	29.0 ± 7.9	24.0 ± 9.6	24.4 ± 9.4	0.09 [V]
FFMI [IV] (kg/m^2)	16.4 ± 2.8	16.3 ± 2.6	16.6 ± 1.8	0.964 [V]
% poor	29.4	35.3	20.0	0.503 [VI]
HGS [IV] kg (N)	30.3 ± 11.9 (38)	27.8 ± 9.6 (18)	27.1 ± 9.8 (16)	0.538 [V]
<P10 [IV] (%)	47.4	66.7	62.5	0.326 [VI]

[I] RCDII lower BMI than CD, [II] EATL more % unintentional weight loss than CD (p=0.04) and trend with RCDII (p=0.082), [III] RCDII trend for higher % FFM and lower kg FM than CD , [IV] Malnourished classified as >10% weight loss (unintentional) in the past 6 months, FM: fat mass, FFM(I): fat free mass (index), HGS: handgrip strength, <P10 according to Bohannon's reference, [V] ANOVA, [VI] Chi-square

Figure 1 depicts an overview of nutritional status with regard to weight loss (unintentionally, >10% in past 6 months) and low BMI (<18.5 kg/m^2) in the three patient groups. FFMI and HGS data did not different between the three groups and, additionally, these were below cut-off values in one-third and two-third of all patients, successively. FFMI and BMI were positively correlated (Pearsons *r* 0,593, p<0.001). But 19% of the patients with a normal/high BMI still had a decreased FFMI and 25% a decreased HGS. Of the 49 patients classified with a normal nutritional status (i.e. no WL and a normal BMI), 21% still had a low FFMI and 17% a low HGS.

Figure 1: Distribution of nutritional status based on combined (unintentional) weight loss and BMI in CD, RCDII and EATL patients



Energy balance

Data on nutritional intake, fecal losses, intestinal absorption capacity, measured REE and calculated energy balance are depicted in **table 3**. No statistical differences were detected between the three groups in energy nor macronutrient intake.

Table 3: Nutritional intake, Resting Energy Expenditure, fecal losses and intestinal absorption capacity (mean ± SD, (range)) of patients with RCDII and EATL versus CD at presentation

	CD novo (43)	RCDII (24)	EATL (25)	p-value
Nutritional intake				
Energy (kcal/d)	2457 ± 660	2882 ± 708	2736 ± 1357	0.205 [V]
Fat (g/d)	88.7 ± 28.3	108.5 ± 38.3	95.5 ± 58.4	0.203 [V]
Protein (g/d)	86.0 ± 26.2	99.7 ± 26.6	95.1 ± 49.5	0.311 [V]
Carbohydrate (g/d)	291.6 ± 89.9	337.3 ± 83.4	338.2 ± 172.6	0.229 [V]
Resting Energy Expenditure				
REE measured (kcal/d) (N)	1596 ± 296	1595 ± 365 (20)	1724 ± 344	0.340 [V]
REE predicted (kcal/d)	(42)	1368 ± 200	(18)	*0.042 [V]*
Measured REE–predicted REE	1512 ± 238	305 ± 68	1413 ± 194	*0.005 [I, V]*
(kcal/d)	196 ± 30	(-208 – +775)	311 ± 303	
% Underestimated REE (>10%)	(-328 – +673)	60.0 %	(-220 – +953)	*0.006 [VI]*
REE/kg (kcal/kg)	38.1 %	26.2 ± 5.5	88.9 %	0.060 [V]
	23.8 ± 4.6		26.8 ± 5.5	
REE/kg FFM* (kcal/kg)	33.3 ± 5.9	33.8 ± 5.3	37.0 ± 8.1	0.174 [V]
Fecal losses and intestinal absorption capacity				
Fecal production (g/d) (N)	242 ± 183 (39)	618 ± 913 (18)	852 ± 2365 (16)	0.196 [V]
$Fecal_{energy}$(kcal/d)	277 ± 137	589 ± 451	506 ± 680	*0.017 [II, V]*
Energy absorption (%)	88.6 ± 5.0	76.9 ± 23.0	83.4 ± 13.2	*0.014 [II, V]*
% Malabsorption (<85%)	21.6 %	44.4 %	33.3 %	0.212 [VI]
$Fecal_{fat}$ (g/d)	8.5 ± 5.4	21.7 ± 20.6	15.8 ± 16.9	*0.003 [III, V]*
Fat absorption (%)	90.2 ± 6.7	77.5 ± 23.2	83.0 ± 11.6	*0.006 [III, V]*
% Malabsorption (<85%)	21.6 %	47.1 %	46.7 %	0.085 [VI]
$Fecal_{nitrogen}$ (g/d)	2.0 ± 0.9	3.2 ± 2.1	3.3 ± 4.0	0.070 [V]
Protein absorption (%)	84.8 ± 7.0	76.4 ± 23.0	73.9 ± 25.1	0.065 [V]
% Malabsorption (<85%)	48.6 %	46.7 %	53.3 %	0.930 [VI]
$Fecal_{carbohydrate}$ (g/d)	35.2 ± 21.4	69.4 ± 60.5	65.2 ± 103.3	0.078 [V]
Carbohydrate absorption (%)	87.1 ± 6.9	77.6 ± 23.9	84.3 ± 15.4	0.096 [V] 0.743
% Malabsorption (<85%)	37.8 %	46.7 %	33.3 %	[VI]
Energy balance				
% Negative (>100 kcal/d)	25.6 %	20.8 %	28%	0.850 [VI]

[I] EATL higher deviation from predicted REE formula than CD, [II] RCDII higher fecal energy loss / lower energy absorption than CD, [III] RCDII higher fecal fat loss and lower fat absorption than CD, [IV] REE: resting energy expenditure, measured with indirect calorimetry or predicted with Harris and Benedict equation, FFM: fat free mass, [V] ANOVA, [VI] Chi-square

Resting energy expenditure

Absolute values of 30 minutes measured REE, did not seem to differ between the three groups, however there were statistical differences in the percentage of patients with predicted underestimation (p=0.006) Measured REE was over 10% higher than the predicted REE in 89% of the EATL patients, with a mean difference of 311 kcal/day, whereas the measured REE was over 10% higher than the predicted REE in 60% of the RCDII patients, representing a mean underestimation of 305 kcal/d. In naive CD patients, REE was underestimated in 38% of patients. REE adjusted for body weight (REE/kg) and FFM (REE/kg FFM) did not differ between the three patients groups, although there was a trend for higher REE/kg body weight in both RCDII and EATL patients (p=0.06). EATL patients had a higher adjusted REE for bodyweight (*t*-test, p=0.036) and a trend for higher FFM adjusted REE (*t*-test, p=0.082), compared to CD patients. Similarly, a statistical trend was seen for the RCDII versus CD patients (*t*-test, p=0.069) on body weight adjusted REE (kcal/kg).

Intestinal absorption capacity

Fecal energy, fat loss and subsequent malabsorption of macronutrients were most aberrant, and therefore disadvantageous, in RCDII patients. A trend was seen for protein and carbohydrate absorption (fecal loss and malabsorption) when comparing RCDII and EATL with naive CD patients, whereas no differences could be detected between RCDII and EATL patients. Severe malabsorption (<75%) was more often present in complicated CD, that is in 17.6-38.9% (depending on energy or macronutrients) of the RCDII patients, 6.7-20% of the EATL patients, but only in 2.7-8.1% of the naive CD patients. When comparing patient cohorts with no malabsorption, malabsorption or severe malabsorption, a statistically significant difference was observed for fat absorption (p=0.025) and a trend for energy absorption (p=0.056). This indicated that naive CD patients more frequently had a normal fat and energy absorption if compared to RCDII and EATL patients. The combination of energy intake, TEE and fecal energy loss resulted in a negative energy balance of >100 kcal/d in 20-28% of all patients (no differences between groups).

Discussion

It is generally accepted that malnutrition and diarrhea may be serious problems in active or naive CD and, more extensively, in its complicated forms, RCDII and EATL. This negatively influences general health, wellbeing, quality of life, morbidity and mortality (28). The present study describes and compares the nutritional status of naive CD, RCDII and EATL patients in a comprehensive way, focusing on anthropometrics, body composition, energy expenditure and muscle strength. In the particular group of RCDII and EATL patients, nutritional status was seriously affected at presentation, even when compared to newly diagnosed, naive CD-patients, as reflected by the high number of patients with involuntary weight loss and loss of muscle mass or muscle strength. This observation may be attributed to a combination of a (unexpectedly) high REE and decreased intestinal absorption. Nutritional status and energy balance are known to be affected in CD patients at diagnosis; in patients with RCDII or EATL, nutritional status may even be described as endangered.

The nutritional status of uncomplicated, naive CD patients, in contrast to that of RCDII or EATL patients, has been studied before, especially with regard to BMI and body composition. Lower body weights, BMI, FM and FFM have been reported (29). Untreated CD patients have a BMI that is approximately 2.3 points lower than that in controls, but this is usually reversible on a GFD. In this study, 30% of the currently diagnosed, naive celiacs even had overweight at presentation, in contrast to 16% of the EATL patients, while overweight was practically not present in RCDII patients (4%). Reported mean BMI in untreated, uncomplicated CD patients is 19.0-24.0 kg/m^2, which is comparable with the observed data in the current CD population (30-32).

Body composition (FM and FFM) of CD patients was studies before, showing a decreased FM in both treated and untreated CD patients if compared to healthy controls (30, 31, 33-36). In this study, the more reliable method FFMI (FFM adjusted for height) was presented and appeared to be impaired in approximately one-third of the naïve CD, RCDII and EATL patients. As expected FFMI and BMI correlated reasonably well; indicating that a higher BMI correlated with a higher FFMI (or vice versa). Besides, it is known that nutritional status has a great impact on muscle strength, a finding which has been corroborated by our hand-grip strength data. Approximately two out of three RCDII or EATL patients had low handgrip strengths, consistent with a negatively affected nutritional status in all studied groups. More remarkably data of this study were that one-fifth of the patients with a normal/high BMI still had a decreased FFMI and a quarter had a

decreased HGS. Of the patients classified with a normal nutritional status, this was 21% and 17%, respectively.

In two prior studies, REE was examined in CD patients (35, 36). REE was found to be higher in patients with CD than in controls, also after adjustment for sex, age and body composition. In the present study, no statistical differences in (uncorrected) measured REE between naive CD, RCDII and EATL patients were observed. However, when comparing measured (objectified) REE to predicted (calculated) REE, the frequently used prediction equation of Harris and Benedict underestimated the measured REE in 60% and 89% of EATL and RCDII patients, respectively. In uncomplicated naive CD patients, 39% was incorrectly predicted (calculated). The applied prediction equation (H&B) therefore seems not to be suitable for these (complicated) CD-patients. An energy expenditure higher than expected appeared to contribute to this incorrect prediction of calculated REE. This may be interpreted as hypermetabolism, which seems a feature of serious disease in EATL patients, and possibly also in RCDII patients.

Remarkably, although malabsorption is frequently linked to (R)CD or EATL in literature, it has not been quantified before. In this study it was present in 20-50% of the various CD patient groups. Fecal energy losses were considerably higher (two-fold) in RCDII and EATL patients than in naive CD patients, i.e. nearly 600 kcal/d versus 277 kcal/d, and subsequent malabsorption (<85%) was prevalent in 44% vs 21% of the two patient groups. Energy loss was accompanied by loss of all macronutrients; overall, and not unexpectedly, energy and macronutrient losses were higher in complicated CD patients, contributing to the clinical characteristics of these illnesses. Patients with complicated CD had a higher fecal production than CD patients, although not statistically significant, due to a large spread and range in relatively small patient groups. In an earlier study we already demonstrated that intestinal function was reduced in patients with CD and RCD, since generation of citrulline out of glutamine was impaired, indicative for a gradual enterocyte mass decline in these patients groups (37).

Previous data on nutritional status in RCDII and EATL patients, including body composition, hand grip strength and energy expenditure, are not available. It has been described that weight loss is common in RCDII patients, also in patients receiving treatment, but a comprehensive assessment of nutritional status has not been described before. One observational study was conducted in a small group of nine RCD patients, showing that weight loss was one of the most common presenting symptoms (38). This is in accordance with the present study in which involuntary weight loss >10% was existing

in 40% of the RCDII patients at presentation. Actually, and in EATL patients in particular, sizeable involuntary weight loss (malnutrition) was one of the most characteristic features (60% of the patients at presentation). On the contrary, RCDII patients were more often characterized by a more chronic form of malnutrition, i.e. a low BMI.

In addition, 52% of EATL patients were homozygote for DQ_2, compared to 37% of the RCDII patients and only 7% of the CD patients. Homozygosis for HLA-DQ_2 has been demonstrated to be related to RCDII and EATL before. Identification of HLA-DQ_2 homozygous CD patients at an early stage, may be helpful to identify the CD patients susceptible for developing these severe complications (39).

A limitation of this study is the relative small sample size of the three groups, although including the largest available series describing nutritional status and intestinal absorption of RCDII and EATL patients. Besides, we realize that the defined fecal carbohydrate content (calculated from the 'remaining fecal energy', thus not explained energy fraction of the stools) was partly inaccurate, since it included indigestible fibres and lactic acid due to ex-vivo fermentation of carbohydrates. As the fecal osmolality had not been measured we could not (semi-)quantify how much these indigestible fibres contributed to the overall assessment.

Conclusion

In conclusion, the nutritional status of RCDII and EATL patients at presentation was seriously disturbed, much more than that of newly diagnosed naive CD patients. Both fecal losses as well as energy expenditure were much higher than expected (described as the phenomenon of hypermetabolism) contributing to (severe) malnutrition at diagnosis. RCDII patients more often had a chronic form of malnutrition, presenting with a low BMI (<18.5 kg/m^2), while EATL patients presented more often with acute malnutrition (substantial weight loss with a BMI >18.5 kg/m^2 and < 25 kg/m^2). In addition, this study may underline the clinical importance of using comprehensive measures of nutritional status and energy balance, specifically in RCDII and EATL patients. The (suspicion of a) diagnosis of RCDII or EATL should therefore initiate an extensive diagnostic nutritional assessment as an incorporated part of the diagnostic work up, medical treatment, and follow-up of these patients.

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